GenØk - Centre for Biosafety

Vår ref:2014/h119
Deres ref: 2014/7565

Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 18.08.14

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet på høringen av søknad **EFSA/GMO/NL/2013/119** som gjelder mat, fòr, import og prosessering av genmodifisert oljeraps **MON88302xMS8xRF3**.

Vennligst ta kontakt hvis det er noen spørsmål.

Med vennlig hilsen,

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GenØk - Centre for Biosafety

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Assessment of the technical dossier submitted under EFSA/GMO/NL/2013/119 for approval of MON88302xMS8xRF3 oilseed rape

Sent to

Norwegian Environment Agency

by

GenØk- Centre for Biosafety
August 2014

KONKLUSJON PÅ NORSK

Vi trekker frem mangler i dossieret som ikke gir grunnlag for en konklusjon om sikker bruk, samfunnsnytten og bidrag til bærekraftighet av MON88302xMS8xRF3 oljeraps. Søker har ikke inkludert noe av den informasjonen omkring samfunnsnytten og bærekraftighet til MON88302xMS8xRF3 oljeraps som kreves i den norske genteknologiloven (Appendix 4) for godkjenning i Norge.

Hovedkonklusjon og anbefalinger
Genøk–Senter for Biosikkerhet viser til brev fra Miljødirektoratet angående høring som omfatter MON88302xMS8xRF3 oljeraps for bruksområdet import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra MON88302xMS8xRF3 oljeraps.

Søker gir ikke opplysninger som adresserer vurderingskriteriene bærekraft, samfunnsnytten og etiske aspekter som forutsettes anvendt i den norske genteknologiloven. I denne sammenheng er det viktig å få dokumentert erfaringer med hensyn på effekter på miljø, helse og samfunnsaspekter. Denne type dokumentasjon er ikke tilstrekkelig i søknaden om omsetting av MON88302xMS8xRF3 oljeraps til import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra MON88302xMS8xRF3 oljeraps.

Vår konklusjon er at norske myndigheter ikke godkjenner bruk av MON88302xMS8xRF3 oljeraps til import og prosessering og til bruk i fòr og mat som det søkes om.

SUMMARY OF THE ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL/2013/119

As a designated National Competence Center for Biosafety, our mission at GenØk in advice giving is to provide independent, holistic and useful analysis of technical and scientific information/reasoning in order to assist authorities in the safety evaluation of biotechnologies proposed for use in the public sphere.

The following information is respectfully submitted for consideration in the evaluation of product safety and corresponding impact assessment of event **MON88302xMS8xRF3 oilseed rape**, setting out the risk of adverse effects on the environment and health, including other consequences of proposed release under the pertinent Norwegian regulations.

In this joint application from Monsanto and Bayer, the Applicant is referring to the molecular data presented in two applications that has already been considered:

- EFSA/GMO/BE/2011/101 for MON88302 (our previous comments from June 2013: H-101)
- EFSA/GMO/BE/2011/81 for MS, RF3 and MS8xRF3 (our previous comments from December 2011: H_81)

Specific recommendations

Based on our findings, we propose a few specific recommendations, summarized here and detailed in the critique below.

- The regulator is encouraged to ask the Applicant to include long term exposure/feeding studies before the GM plant product is released on the marked for food/feed consumption.

- The regulator is encouraged to ask the Applicant to consider that we find that it would be ethically incongruous and a double standard of safety for Norway to ban the use of these herbicides domestically as a health concern, but support its use in other countries.

- The regulator is encouraged to ask the Applicant to demonstrate the lack of interactive effects between transgenic proteins through proper scientific testing and evidence gathering, rather than justify the lack of testing based on assumptions-based reasoning of no effects.

- The regulator is encouraged to ask the Applicant to provide data on genetic and phenotypic stability of the GM plant that is been assessed, i.e. the stack event MON 88302 X MS8 X RF3

- The regulator is encouraged to ask the Applicant to provide data on genetic and phenotypic stability of inserts under different environmental and abiotic stress conditions.

- The regulator is encouraged to ask the Applicant to conduct analysis on genome stability of the GM plant in comparison to the parental conventional counterpart. The genome stability should be conducted under different environmental and abiotic stress conditions

- The regulator is encouraged to ask the Applicant to conduct detailed metabolomics, transcriptomic and proteomic analysis in which the analyses are targeted to GROUPS of metabolites or anti-nutrients that may be affected by the genetic modification

- The regulator is encouraged to ask the Applicant to take measures to ensure future coexistense of non-transgenic cultivars by eliminating the risk of both accidental spillage and contamination of transport equipment

Application: EFSA/GMO/BE/2011/101 for MON88302 (our previous recommendations from June 2013: H-101)

- The regulator is encouraged to ask the Applicant to use a set of smaller probes and used a labeled marker in the upset.

- In general, the presented figures are acceptable. However, the southern blot figures lack a visible molecular weight marker on the membrane which should always be present.

- Given the deletions reported after integration of the transgenic DNA into the host genome, the Applicant should provide a survey of the actual RNAs produced or absent at the integration junctions and in the DNA surrounding the insert, preferably using high throughput transcriptome sequencing techniques (Heinemann et al 2011).

- The methods used in the expressions studies are not detailed enough in order to make an appropriate evaluation. The expression levels of CP4 EPSPS show high variation between plants. Whether this might have an effect on the glyphosate tolerance or the forage quality is not predictable but should be examined. In addition it is not specified if the used antibodies were raised against CP4 EPSPS protein derived from E. coli or against the MON88302 CP4 EPSPS.

- In general, there is no scientific literature available on the genetic construct, the genetic stability, transgene expression products or immune-toxicological effects, in order to make an appropriate scientific evaluation.

- The protein is expressed in a new context in this event and should be analysed more thoroughly as it differs from the previous events where this protein is expressed.

- The Applicant should use the plant produced CP4 EPSPS and not the *E.coli* produced CP4 EPSPS to assess the safety of the protein

- The figures presenting western blots of the protein should have visible molecular weight markers, and not only arrows indicating sizes.

- Activity of CP4 EPSPS protein isolated from representative food and feed should be analysed

- The Applicant should use the plant produced CP4 EPSPS and not the *E.coli* produced CP4 EPSPS to assess the safety of the protein.

- The Applicant should address the issue of potential allergenicity by testing the representative feed/food material in animal and human allergenicity testing models.

- The Applicant should use the plant produced CP4 EPSPS and not the *E.coli* produced CP4 EPSPS to assess the safety of the protein.

- The Applicant should include non-rodent species as test organisms for the toxicity studies.

- The Applicant should include a long-term feeding study in the toxicological testing

EFSA/GMO/BE/2011/81 for MS, RF3 and MS8xRF3 (our previous recommendations from December 2011: H-81)

- The applicant's submission, and its interpretations should be conducted on the basis not only as "accidental, unintentional presence" in food, but as if the event applied for use in food was to be wholly consumed at any level of consumption. The application should be analyzed in such a manner that conforms not to the smallest level of exposure that would be allowed with the approval, but the largest.

- The applicant should submit newly designed toxicity and allergenicity studies relevant to the application at hand.

- The applicant should provide information pertaining to the functional status of the transgenic protein after processing and also on the effects of MS8, RF3 AND MS8XRF3 inhalation in animals that are used as models of acute respiratory syndrome, compared with inhalation of the proper conventional comparator. This should include an analysis of allergenicity and toxicity.

- The regulator is encouraged to ask the Applicant to submit required information on the social utility of **MON88302xMS8xRF3 oilseed rape** and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

Overall recommendation

From our analysis, we find that the deficiencies in the dossier do not support claims of safe use, social utility and contribution to sustainable development of **MON88302xMS8xRF3 oilseed rape. Critically, the Applicant has not included any of the required information to assess social utility and sustainability as required in Appendix 4 of the Norwegian Gene Technology Act, which would be necessary for consideration of approval in Norway.** Hence at minimum, the dossier is deficient in information required under Norwegian law. A new application or reapplication should only be reconsidered with the delivery of the information requests recommended here, including any additional information deemed significant by the Norwegian authorities.

Therefore, in our assessment of **MON88302xMS8xRF3 oilseed rape**, we conclude that based on the available data supplied by the Applicant, the Applicant has not substantiated claims of environmental safety satisfactorily or provide the required information under Norwegian law to warrant approval in Norway at this time.

ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL/2013/119

About the event

The genetically modified MON88302xMS8xRF3 oilseed rape is produced by crossing MON88302 and MS8xRF3 parental lines using conventional breeding methods. MS8xRF3 was obtained by conventional breeding of two single event oilseed rape products: MS8 and RF3. Genetic modification was used in the development of MON88302, MS8 and RF3. All events were developed through *Agrobacterium tumefaciens* mediated transformation.

MON88302xMS8xRF3 oilseed rape produces: the *cp4 epsps* protein which confers tolerance to glyphosate, the *PAT* protein which confers tolerance to glufosinate, the *Barnase* protein which breaks down RNA in pollen and the *Barstar* protein that inactivates the barnase-protein.

The Applicant is requesting the authorization for food, feed, import and processing in the EU of glyphosate tolerant MON88302xMS8xRF3 oilseed rape.

Assessment findings

Herbicides

Glyphosate tolerance

The **MON88302xMS8xRF3 oilseed rape** contains the *CP4EPSPS* gene from *Agrobacterium tumefaciens strain ABI* that confers tolerance to herbicides containing glyphosate.

Glyphosate has been heralded as an ideal herbicide with low toxicity for operators, consumers and the environment surrounding agriculture fields (Duke & Powles 2008, Giesy et al 2000), but has received more risk-related attention due to its negative effects on both aquatic and terrestrial ecosystems (Blackburn and Boutin 2003, Ono et al 2002, Solomon and Thompson 2003) and studies in animals and cell cultures indicate possible health effects in rodents, fish and humans (Marc et al 2002, Axelrad et al 2003, Dallegrave et al 2003, Jiraungkoorskul et al 2003, Richard et al 2005, Benachour et al 2007, Gasnier et al 2009)

Glyphosate kills plants by inhibiting the enzyme 5-enolpyruvoyl-shikimate-3-phosphate synthase (EPSPS), necessary for production of important amino acids. Some microorganisms have a version of EPSPS that is resistant to glyphosate inhibition. The transgene, cp4 EPSPS, used in genetically modified crops was isolated from an Agrobacterium strain. The whole idea is the combined use of the GM plant and the herbicide. Recent studies indicate that agriculture of GM plants is associated with greater overall usage of pesticides than the conventional agriculture (Benbrook 2009). Large

proportions of GM agriculture is glyphosate tolerant crops (GT-cultivars) (James 2010).

A restricted number of recent publications indicate unwanted effects of glyphosate on health (Dallegrave et al 2003, Malatesa M et al 2002), aquatic (Solomon K & Thompson D 2003) and terrestric (Ono MA et al 2002, Blackburn LG & Boutin CE 2003); organisms and ecosystems. Some of these may be considered "early warnings" of potential health and environmental risks, and they should be rapidly followed up to confirm and extend the findings.

Studies in animals and cell cultures point directly to health effects in humans as well as rodents and fish. Female rats fed glyphosate during pregnancy demonstrated increased fetal mortality and malformations of the skeleton (Dallegrave E et al 2003). Mice fed GE soybean demonstrated significant morphological changes in their liver cells (Malatesta M et al 2002). The data suggested that EPSPS-transgenic soybean intake was influencing liver cell nuclear features in both young and adult mice, but the mechanisms responsible for the alterations could not be identified by the experimental design of these studies. Treatment with glyphosate (Roundup) is an integrated part of the EPSPS-transgenic crop application. Nile Tilapia Oreochromis niloticus) fed sublethal concentrations of Roundup exhibited a number of histopathological changes in various organs (Jiraungkoorskul W et al 2003). A study of Roundup effects on the first cell divisions of sea urchins (Marc J et al 2002) is of particular interest to human health. The experiments demonstrated cell division dysfunctions at the level of CDK1/Cyclin B activation. Considering the universality among species of the CDK1/Cyclin B cell regulator, these results question the safety of glyphosate and Roundup on human health. In another study (Axelrad JC et al 2003) it was demonstrated a negative effect of glyphosate, as well as a number of other organophosphate pesticides, on nerve-cell differentiation. Surprisingly, in human placental cells, Roundup is always more toxic than its active ingredient. The effects of glyphosate and Roundup were tested at lower non-toxic concentrations on aromatase, the enzyme responsible for estrogen synthesis (Richard S et al, 2005). The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation. The authors conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. They suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation. In the highly controversial study by Seralini et al (Seralini et al 2012) the authors concludes that long term exposure of lower levels of complete agricultural glyphosate herbicide formulations, at concentrations below official set safety limits, induce severe hormone-dependent mammary, hepatic and kidney disturbances in rats. In a recently published study by Bohn et al (Bohn et al 2014) the authors recommend to focus also on pesticide residues in major crop plants which may have consequences for human and animal health.

Recommendation:

- Long term exposure/feeding studies should be included in a risk assessment before a GM plant product is released on the marked for food/feed consumption.

Glufosinate-ammonium tolerance

The *pat* gene derived from *Streptomyces hygroscopicus* confers tolerance to herbicides containing glufosinate-ammonium, a class of herbicides that are banned in Norway and in EU (except a limited use on apples) due to both acute and chronic effects on mammals including humans. Studies have shown that glufosinat ammonium is harmful by inhalation, swallowing and by skin contact and serious health risks may result from exposure over time. Effects on humans and mammals include potential damage to brain, reproduction including effects on embryos, and negative effects on biodiversity in environments where glufosinate ammonium is used (Hung 2007, Matsumura et al. 2001, Schulte-Hermann et al. 2006, Watanabe and Sano 1998). According to EFSA, the use of glufosinate ammonium will lead to exposures that exceed acceptable exposure levels during application.

The **MON88302xMS8xRF3 oilseed rape** is tolerant to the active herbicide ingredient glufosinate-ammonium through the insertion of the *pat* gene, but it is not portrayed as such in the dossier. The tolerance to herbicide in general is connected to heavier use, and in most cases biotransformation of the herbicides active ingredient to another compound which should also be evaluated for toxicity. Though the mechanism of PAT is described in the dossier, the food and feed safety and toxicology testing, of the references to such tests in other transgene plants has been done without considering the effects of applying glufosinate to the plants.

Recommendation:

- The regulator is encouraged to ask the Applicant to consider that we find that it would be ethically incongruous and a double standard of safety for Norway to ban the use of these herbicides domestically as a health concern, but support its use in other countries.

Stacked events

Until recently, the dossiers submitted for marked authorization almost only covered single GM events. Today there is a clear trend to combine two or more transgenic traits present in single events through traditional breeding. However, information on how these GM stacked events should be assessed is limited and in some cases assessment data for each single GM events has been taken into account to prove the safety of the whole food/feed.

Stacked events are in general more complex and it has been an increased interest in the possible combinatorial and/or synergistic effects that may produce unintended and undesirable changes in the plant – like the potential for up- and down regulation of the plants own genes. Interactions with stacked traits cannot be excluded that the group of expressed toxins in the plant can give specific immunological effects or adjuvant effects in mammals (Halpin 2005, deSchrijver et al, 2007). Then (2009) reviews and discusses the evidence for changes in activity and specificity of Bt proteins dependent on synergistic interactions with extrinsic features. Such changes may critically influence the bioactivity and hence the potential for unintended effects.

Most of the information submitted in this safety assessment is derived from previous finding with the single lines. In general the applicant describes most of the traits and characteristics of the "stacked event" as being the same as those of the parental GM events used in production of GM soybeans. That applicant has not demonstrated that interactions among the different transgenic proteins, particularly for allergenic or toxic effects, are not taking place in this event, despite evidence of the potential effects? (Mesnage et al., 2012). Assumptions-based reasoning with single events should not replace scientific testing of hypotheses regarding interactions. GenØk means that stacked events cannot be approved based on the information on the single events.

MON88302xMS8xRF3 oilseed rape combines three different genetically modified oilseed rape. This is why combinatorial, synergistic effects must be carefully considered in the development and risk assessments of stacked events and robust data are necessary to identify whether the combined presence of transgenes influences expression levels, e.g. by silencing effects.

Recommendation:

- The regulator is encouraged to ask the Applicant to demonstrate the lack of interactive effects between transgenic proteins through proper scientific testing and evidence gathering, rather than justify the lack of testing based on assumptions-based reasoning of no effects.

Information relating to the genetic modification (p 17)

The Applicant states that a description of the genetic modification of the single parental events in **MON88302xMS8xRF3 oilseed rape** has been previously provided in the EFSA/GMO/BE/2011/101 and EFSA/GMO/BE/2011/81 applications.
Therefore, the comments about molecular characterization for MON88302xMS8xRF3 oilseed rape are also based on the single parental events (MON88302 and MS8xRF3).

Application: EFSA/GMO/BE/2011/101 for MON88302 (our previous comments from June 2013: H-101)

PCR and Southern Hybridization

The Applicant states: "The molecular characterization of MON88302 does not raise any safety concern and does not show any evidence of unintended changes in MON 88302."
Characterization of the DNA insert in MON88302 was conducted by Southern blot, PCR and DNA sequence analyses.

Insert and copy number

To test for the numbers of copies and insertion sites of the T-DNA sequences in the oilseed rape genome, restriction enzymes and Southern blot hybridization was used. The Applicant have used probes for southern blot hybridization ranging in size from 1,3-2,3 kb (listed in figure 2 p 26). The use of long probes to detect recombinant DNA can lead to false negative results. The strength of the interaction between probe and target is based on the number of bonds that form between the single strand of DNA that is the probe and the matching recombinant DNA that is the target. A long probe that binds perfectly to a short insertion will not be strongly bound and may be washed off depending on the stringency of the wash. The best probe is one that approximates the size of the target sequence and does not exceed approximately 500 nucleotides in length. Probes that are > 500bp means that point mutations, small deletions and rearrangements that might occur during breeding will possible not be detected (Fagard&Vauvheret 2000, de Schrijver et al 2006). This means that in this case, the applicant failed to account for potential inserts that are only partial, either smaller than the probes or with rearrangements, both of which could prevent binding of the probe and therefor detection of rDNA integrated elsewhere in the genome (Kononov et al 1997).
In general, the southern membranes provided in the dossier and also in some of its reports lack labeled markers. How can you know that the band has the expected size without using a marker?

Recommendation:

- The applicant should use a set of smaller probes and used a labeled marker in the upset.
- In general, the presented figures are acceptable. However, the southern blot figures lack a visible molecular weight marker on the membrane which should always be present.

Detection of absence of backbone vector DNA/unintended transgenes

Examination of the insert, the flanking genomic and genomic DNA insertion site was characterized by PCR and DNA sequencing. A 9 base pair insertion adjacent to the 3`end of

the MON88302 insert, a 29 base pair deletion from the conventional genomic DNA occurred during the insertion of the T-DNA into the conventional oilseed rape to form MON88302, and a single nucleotide difference between the conventional counterpart sequence and the known DNA sequence flanking the 3`end of the MON88302 insert was reported by the Applicant.
The Applicant states that these molecular rearrangements presumably resulted from double-stranded break repair mechanisms in the plant during the agrobacterium-mediated transformation process (Salomon and Puchta 1998), however they do not mention any possible consequences because of these rearrangements.

Recommendation:

- Given the deletions reported after integration of the transgenic DNA into the host genome, the Applicant should provide a survey of the actual RNAs produced or absent at the integration junctions and in the DNA surrounding the insert, preferably using high throughput transcriptome sequencing techniques (Heinemann et al 2011).

Genetic stability of the insert and phenotypic stability of the GM plant

The Applicant deduced phenotypic stability of the ***GM plant*** from the molecular characterization of MON 88302 X MS8 X RF3. Data presented showed the GM plant retained the MON 88302, MS8 and RF3 inserts with their inherent properties, (Section 2.2.4, page 40 of Applicant's dossier). However, retention of the respective inserts and their functions confirms *only phenotypic stability of the inserts but not the phenotypic stability of the entire GM plant*. We note also that the stability analyses that for the event under consideration (i.e. MON 88302 X MS8 X RF3) was not conducted, instead data based on the analyses of single events of MON 88302, MS8 and RF3 were presented; even so from another variety (the Ebony canola variety). The Applicant concluded "… *there is no scientific basis to support the notion that these sequences would be intrinsically more unstable when combined together by conventional breeding. Therefore it is appropriate for the MON 88302 X MS8 X RF3 molecular characterization to refer to the molecular characterization of MON 88302, MS8 and RF3*." The study of Skottke et al., (2013) referenced by the Applicant was a study to confirm the presence of MON 88302, MS8 and RF3 inserts in the combined traits of MON 88302 X MS8 X RF3. It did not prove genetic and phenotypic stabilities of these inserts in the new GM plant. Our opinion is that the Applicant has not demonstrated sufficient and rigorous scientific testing, but has based his conclusion on assumptions.
In section 3.4.2 (page 68 & 69), the applicant provided data on differences in phenotypic characteristics between MON 88302 X MS8 X RF3 (Not Treated & Treated) and the conventional control as well as equivalence between MON 88302 X MS8 X RF3 and a set of conventional reference varieties for selected 11 characteristics (Not Treated & Treated) (Tables 10, 11 & 12). In the context of risk assessment, phenotypic stability should be differentiated from substantial equivalence between the GM plant and parental conventional counterpart. To show phenotypic stability in the stacked events (i.e. in the GM plant), additional comparators, namely, the single events of MON 88302, MS8 and RF3 are required to provide robust data on the phenotypic stability of the new GM plant. Further, the MON 88302 X MS8 X RF3 GM plants from which the transgenes have been removed (i.e. a revertant variety) should also serve as a comparator. This comparator would provide a basis for understanding the combined effects of the genetic manipulations and conventional

breading technology given that the latter also has effect on plant phenotypes and can lead to unwanted adverse effects (Baudo et al., 2006).
The applicant, in Section 3.4.3 (page 69), provided information on environmental interaction assessments conducted as part of the plant characterization of MON 88302 X MS8 X RF3 (Moon et al., 2013a). However, the Applicant did not state why the herbicide treated MON 88302 X MS8 X RF3 (T) was excluded from this study. Thus, the inserts stability of the biotechnology derived traits (glufosinate-ammonium and glyphosate tolerance) of MON 88302 X MS8 X RF3 during in-crop applications of glufosinate and glyphosate was not determined. An environmental interaction assessment in which the MON 88302 X MS8 X RF3 (T) is included will provide a more robust evaluation for risk assessment purposes.

Furthermore, the Applicant assessed genetic stability of MON 88302 X MS8 X RF3 by confirming the presence of inserted sequences of MON 88302, MS8 and RF3, and showed that no detectable rearrangements of these inserts occurred using Southern blot analyses. We have stated our concern with the probe design under ***"PCR and Southern Hybridization"*** section (page 13) of this report. However, while the Applicant has provided data on genetic stability of MON 88302 X MS8 X RF3 following breeding of MON 88302, MS8 and RF3. Data showing that the transgenes are stable under various environmental and abiotic stress conditions and over several generations were not provided. In the least the Applicant should clearly state that the data presented confirm genetic and phenotypic stability of only the inserts. In addition, the genetic and phenotypic stability data are for a single generation.
Genetic stability of inserts is different from genome stability of the GM plant. Genome stability will reveal, in comparison to the parental conventional comparator, that there are no changes in the genome of the GM plant following genetic modification. Southern blot analysis used by the Applicant is a targeted approach that can reveal only inserts' genetic stability but not genome stability. The Applicant has not provided data on genome stability analyses of the GM plant under different environmental and abiotic stress conditions and over different plant generations. As an example, the genome stability of the GM event in comparison to the parental counterpart can be determined by deep sequencing of transcriptome or RNA (RNA-seq).

Recommendations:

- The Applicant should provide data on genetic and phenotypic stability of the GM plant that is been assessed, i.e. the stack event MON 88302 X MS8 X RF3. Data should not be based on assumptions, but on rigorous scientific and evidenced based testing.
- In the least the Applicant should clearly state that the data presented confirm genetic and phenotypic stability of only the inserts. In addition, the genetic and phenotypic stability data are for a single generation.
- The Applicant should provide data on genetic and phenotypic stability of inserts under different environmental and abiotic stress conditions.
- The Applicant should conduct analysis on genome stability of the GM plant in comparison to the parental conventional counterpart. The genome stability should be conducted under different environmental and abiotic stress conditions.

ELISA

The applicant states: "The CP4 EPSPS protein expression levels (µg/g dw) determined from treated tissues of MON 88302 were comparable to those determined from untreated MON 88302 tissues, showing that glyphosate application in MON 88302 does not alter nor have any negative effects on the expression of the CP4 EPSPS protein in the plant." Information on the expression of the inserted modified sequence was conducted by ELISA.

While the mean CP4 EPSPS expression levels in untreated and glyphosate treated Mon 88302 are comparable in forage and grain there is a wide range of expression levels 120-210 µg/g dwt and 90-290 µg/g dwt for sprayed and unsprayed forage and 22-46 µg/g dwt and 22-42 µg/g dwt for spayed and unsprayed grain. Sprayed over- season leaves even had an expression range between 110 and 500 µg/g dwt. The expression levels of CP4 EPSPS show high variation between plants. Whether this might have an effect on the glyphosate tolerance or the forage quality is not predictable but should be examined.
Further information about the time difference between the last glyphosate treatment and the sampling of the plant material is important to interpret the CP4 EPSPS expression levels.

The description of the ELISA method in the given references (Clark 2012a; Clark and Niemeyer 2010a) is not detailed enough. Important information necessary to replicate the measurements is missing like a detailed description of the protein extraction method, antibody dilutions and how many parallels of each sample that were measured. Additionally the raw data (OD-values) and standard deviations for sample parallels are not available. The inter-assay negative and positive controls are not specified.

Under point 4.6 and 5.1 as well as point 2 below table 1 of the Clark and Niemeyer 2010a report it is mentioned that samples which showed unexpected negative results or "unexpected results" during ELISA or PCR were omitted. It is not further explained what is considered an "unexpected result" or why these were excluded.

As a protein standard *E.coli* produced CP4 EPSPS protein was used. Since there might be differences in the affinity of the used antibodies for *E.coli* and Mon 88302 derived CP4 EPSPS the Mon 88302 derived CP4 EPSPS should be used for a standard curve. Figure 20. Molecular weight and purity analysis of the MON 88302-produced CP4 EPSPS shows that it was possible to purify quite high amounts of the protein and therefore it should be used as a standard in the ELISA.

Furthermore it is not specified if the used antibodies were raised against CP4 EPSPS protein derived from an *E.coli* expression system or against the Mon 88302 CP4 EPSPS. Antibodies raised against the *E.coli* derived CP4 EPSPS might in fact not be able to detect all isoforms of the Mon 88302 CP4 EPSPS possibly produced in-planta.

Recommendation:

- The methods used in the expressions studies are not detailed enough in order to make an appropriate evaluation. The expression levels of CP4 EPSPS show high variation between plants. Whether this might have an effect on the glyphosate tolerance or the forage quality is not predictable but should be examined. In addition it is not specified if the used antibodies were raised against CP4 EPSPS protein derived from E. coli or against the MON88302 CP4 EPSPS.
- In general, there is no scientific literature available on the genetic construct, the genetic stability, transgene expression products or immune-toxicological effects, in order to make an appropriate scientific evaluation.

Health effects

Regarding potential health effects the applicant claims safe use since oilseed rape in general has a long history of safe use. The applicant also claims that the event is not considered to have toxic effects to humans, animals and other organisms.
However, the data provided in the dossier do not give enough evidence that the use of MON88302 is safe from a toxicological nor allergenic point of view. No scientific studies on the plant variety in question are available in order to make an appropriate scientific evaluation.

No feeding studies have been performed in animals. No studies of allergenicity have been performed in neither animals nor humans. The applicant states that such testing is "not necessary", since the non-toxicological and non-allegenic properties of the CP4 EPSPS protein are well established (sections A 4.2.5, A 4.5 and A 5.4). Thus the applicant ignores a main point of the EFSA-risk assessment guidelines for GM-plants, namely that the risk of unwanted or adverse effects due to changes in the recipient genome must be anticipated and tested. Thus, it is not only a question of effects directly produced by the inserted CP4 EPSPS gene, but also important to evaluate indirect effects stemming from the modification itself. Here EFSA is very clear and recommends animal feeding trials to address this specific risk.

Toxicological assessment
The assessment of potential toxicity of the expressed CP4 EPSPS protein is included in the dossier with the same arguments as previous GM plants with the same inserted gene. These arguments are the ones that are most commonly used:

- History of safe use
- No structural similarity to known toxins
- No acute toxicity effects to mammals
- Rapid digestion in digestive fluid

The argument of long history of safe use is based on the "fact" that this bacterially derived protein has posed no risk to human health since its introduction to food and feeds in 1996 (Delaney et al 2008) and that the actual concentration of the CP4 EPSPS protein is very low in food and feed. The applicant has not analysed the question of safety further because of the "weight of evidence" provided in the long history of safe use. Still, the protein is expressed in

a new context in this event (expression throughout the plants development) to be able to spray it with glyphosate herbicides more frequent and at an earlier stage and should be analysed more thoroughly as it differs from the previous events where this protein is expressed.

Recommendation:

- The protein is expressed in a new context in this event and should be analysed more thoroughly as it differs from the previous events where this protein is expressed.

Assessment of the newly expressed protein

For the assessment studies, the applicant uses *E.coli* produced CP4 EPSPS as "the levels of introduced protein *in planta* usually are too low". However, they use the plant version of the protein in the molecular weight and protein purity assays, thus they are able to isolate the protein. The bacterial version is used for *in vitro* digestibility, acute oral toxicity and heat stability analysis of the protein.

The applicant provides evidence for equivalence between plant and bacterial version of CP4 EPSPS. However, the applicant should search to use the plant version of the protein in these analyses to get the most authentic results as the two proteins are expressed in bacteria and plant. This means that the protein that actually is expressed in the gene modified species, and derived from it, should be used due to the potential differences that can arise because of post translational differences between species, tissues and stages of development (Gomord et al 2005, Küster et al 2001).

The plant and the bacterially derived proteins seem to be of same size and immune-reactivity based on the results presented by the applicant. The acceptation criteria for immune-reactivity are set to +/- 35 %. These are met (24.1 % average difference is presented. The difference varies between 14.9 and 30.8 percent, depending on the concentration of the protein loaded in the gel). Higher concentrations of protein in gel gives more saturated bands and thus they are measured more equal in concentration. It therefore seems important to have suboptimal ag/protein levels in the gel to get real comparative data (bands that are not saturated).

Figure 21, showing the western blot analysis of plant and bacterially derived CP4 EPSPS protein should have been exposed longer to check the presence of additional bands caused by potential post translational activities and proteins of different size. One would also assume that the antibody used in the immunogenic reactions are raised using the bacterially derived version of the protein, raising another issue on equality and reactivity of proteins expressed from as different sources as plants and bacteria.
There should also have been a presentation of the result after protein isolation from representative food and feed containing MON88302, to verify presence/absence and immune-reactivity.

The bacterial and the plant derived CP4 EPSPS proteins were analysed for the presence of glycosylation as many eukaryotic proteins are post-translationally modified with carbohydrates (Rademacher et al 1988) while prokaryotic glycosylation is less common. The presence of glycosylation has also been used as one of the criteria for a potentially allergenic protein as allergenic proteins often are found to be glycosylated. No glycosylation was

detected of the plant version of CP4 EPSPS with the method used. The positive control is visible after 2 min exposure (Figure 5, Bhakta et al 2010). The bands presented in this figure are however quite faint. An additional figure should have been present, indicating if there are any changes to the detected signals when the membrane is exposed further/longer. The applicant uses CP4 EPSPS from bacteria as the negative control. A second negative protein should also have been added, that is not EPSPS.
The plant derived protein also shows no homology to known toxins or biologically active proteins using bioinformatics tools.

Protein stability during processing and storage (food/feed from the gene modified plant) was analysed by performing heat treatment of purified *E.coli* derived protein. The results indicate that the protein loses its activity at high temperatures, also at the relevant temperature of 75°C and higher (processing: conditioning of oil seed rape starts at 75°C). Thus they conclude that the rape seed oil does not contain the protein in question and at least not active (Dossier, p.120). But they have not actually tested the *in planta* version at the relevant temperatures, or the potential food/feed itself. What they do show is that the *E.coli* derived CP4 EPSPS protein maintains its size after the 95°C for 30 min treatment and that it loses its activity at 75°C.

The applicant has also tested the CP4 EPSPS proteins for resistance to protelytic cleavage and intactness when subjected to different pHs. The protein is demonstrated to be intact at neutral and acidic pHs. Also, the protein is rapidly degraded in simulated gastric fluids, yet another indication of low potential as an allergen or toxin. However, they have only tested the bacterial version and not the one found in plants.
In general, the presented figures are acceptable. However, some of them lack a visible molecular weight marker on the membrane (Figures 21, 22, 26).

Recommendation:

- The Applicant should use the plant produced CP4 EPSPS and not the *E.coli* produced CP4 EPSPS to assess the safety of the protein
- The figures presenting western blots of the protein should have visible molecular weight markers, and not only arrows indicating sizes.
- Activity of CP4 EPSPS protein isolated from representative food and feed should be analysed

Assessment of new constituents other than proteins

As a stacked event MON88302xMS8xRF3 has new and complex traits different from the individual respective events of MON88302, MS8, and RF3. At the moment there do not exist appropriate methodologies for comprehensive risk assessment of stacked events. Therefore, instead of relying solely methods and data that were applied for the assessment of single un-stacked events, robust profiling data on MON88302xMS8xRF3 event should be submitted to support data derived from specific targeted approaches. A semi-targeted profiling data would characterize new molecules (e.g. RNA, protein, metabolites) or anti-nutrients at significant concentrations. This way potential unintended effect can be identified and evaluated for possible adverse effects (Van Aggelen et al., 2010).

The applicant has presented data on effects of genetic modification of MON88302xMS8xRF3 on pre-determined endpoints of plant compositional analysis as well as on possible health effects, toxicity and allergenicity. This specific approach captures only information on unintended effects within a narrow biological scope. Therefore, the statement by the applicant on page 81, section 4.3 that "no testing of any constituent other than the introduced new protein is required" because of "long history of safe use and consumption around the world" is a narrow and unscientific assumption. A more robust profiling approach targeting GROUPS of metabolites or anti-nutrients that may be affected by the genetic modification would provide more robust data for risk assessment; this approach can also detect relevant qualitative changes (Reimer et al., 2004). Any changes should then be further investigated in relation to relevant risk assessment parameters. It should be noted that a failure to detect difference is not a proof of absence of differences (and by extension not a proof of safety) (Heinemann et al., 2011).

Recommendation:
Experimental designs that incorporate robust semi-targeted approaches alongside the employed targeted approaches are required to substantiate the claim of absence of new constituents such as anti-nutrients and toxins. Detailed metabolomics, transcriptomic and proteomic analysis in which the analyses are targeted to GROUPS of metabolites or anti-nutrients that may be affected by the genetic modification would provide more robust data for risk assessment should be conducted.

Allergenicity assessment
Allergenicity of the CP4 EPSPS protein is tested through Codex Alimentarius, 2009 (Codex, 2009). The assessment is heavily based on that the protein is from a non-allergenic source, has no structural similarities to known allergens, is rapidly digested and not stable at heat treatment. The conclusion from the applicant is that the protein is not allergenic.

The Applicant does not discuss potential allergenicity of the plant derived version of the protein, but rely on data obtained from the bacterial version of it. Also, the statement that the protein is not stable is not true: the protein is stable up to 95°C and for the tested 30 min (Figure 24). Low percentage of the CP4 EPSPS protein as compared to total protein is presented as one of the points in the allergenicity assessment. However, this is not relevant when it comes to allergenicity as only traces of allergenic protein in food have been found to give allergenic reactions. Interestingly, they are able to isolate protein from rape seed oil in this part of the dossier, although at low levels. This protein is however not analysed further. Only the *E.coli* version of the CP4 EPSPS protein is used for the allergenicity assessment.
The Applicant also states that oilseed rape not is considered to be an allergenic plant. Very few in the population are allergic to oilseed rape plant and pollen. However, oil seed rape with CP4EPSPS is "new" in this context and should be assessed as such.

Analysis of the adjuvancy of the CP4 EPSPS protein has also been performed and no similarity to known strong adjuvants is found. Also, bioinformatic analysis does not find it similar to known allergens.

A major point in this assessment of toxicity and allergenicity is that the applicant uses a different form of the protein than the one actually present in the food/feed they want approved: namely the E.coli version of the CP4 EPSPS protein and not the authentic plant version of it.

The applicant states that; *" There have been a limited number of reports citing oilseed rape flour as an allergen. These studies reported that the four individuals with hypersensitivity to oilseed rape flour worked with animal feed preparation where oilseed rape flour is a component, suggesting the prevalence is low and confined to occupationally exposed populations."* (A 5) and further. *"The incidence of oilseed rape hypersensitivity in the occupationally exposed population, i.e., scientists or farm workers that handle the plant and the pollen on a daily basis, was 31%, but most of these individuals were hypersensitive to multiple allergens"* (A 5).

In section A 5.4 the applicant concludes that they have evidence that the CP4 EPSPS protein is not likely to be allergenic and thus the food (or feed) derived from MON 88302 also is not likely to be more allergenic than other varieties of oil seed rape. This claim is scientifically unjustified and should be rejected.

Thus we must conclude that the applicant does present evidence for potential allergenicity of oil seed rape flour, but does not address this potential effect for this relevant variety in a scientifically acceptable way. The applicant thus should perform relevant testing of MON 88302 allergenicity potential in animal and human allergenicity testing models.

Recommendation:

- The Applicant should use the plant produced CP4 EPSPS and not the *E.coli* produced CP4 EPSPS to assess the safety of the protein.
- The Applicant should address the issue of potential allergenicity by testing the representative feed/food material in animal and human allergenicity testing models.

Feeding experiment

In the dossier presented by the Applicant, no treatment related adverse-effects were observed in animals dosed with CP4 EPSPS protein except from a few minor pathological findings in female mice were observed at necropsy to be randomly distributed among all groups and are commonly seen in the strain of mice used (Harrison et al 2006). Besides, this specific study by Harrison et al is on GT-soybean and has little, if any, relevance to the GT-oil rape in question here. However a number of issues create uncertainty regarding the claim of safety.

First, in this study the *E.coli* produced CP4 EPSPS protein was used to assess the safety of CP4 EPSPS and not the plant-produced proteins. The reason why the applicant prefer the bacterial version is because the levels of introduces proteins in planta are usually too low to allow purification of sufficient quantities for use in safety assessment studies. By using the bacterial version of the protein excludes information on the toxicological potential of the

protein in a genetically modified plant. One should always utilize the version present in the plant as mentioned before (Codex work on Foods derived from Biotechnology, CAC/GL 44-2003, p. 14 and 22).

Second, acute oral toxicity studies may detect large effects, yet have little relevance for substances or products which will be fed or consumed over a lifelong period and exhibit chronic effects. Certain toxicological properties will only become evident in case of systematic testing (Spök et al 2004, 2005). With the acute toxicity study, mice and rats are the normal test organisms, but they should also include one non-rodent species (e.g. dogs) for sub-chronic testing. Proper hazard characterization of any effects noted in these studies may require determining mode of action (EFSA, 2008a).

Recommendation:

- The Applicant should use the plant produced CP4 EPSPS and not the *E.coli* produced CP4 EPSPS to assess the safety of the protein.
- The Applicant should include non-rodent species as test organisms for the toxicity studies.
- The Applicant should include a long-term feeding study in the toxicological testing

Application: EFSA/GMO/BE/2011/81 for MS, RF3 and MS8xRF3 (our previous comments from December 2011: H_81)

We note that previous assessments by competent authorities or expert committees in the EU and Norway do not share the conclusions made by the applicant. We also wish to orient you towards two critical pieces of information related to this assessment, focused on health and toxicological consequence of use of this product and its co-products (e.g. herbicides) for its intended use.

First, the Austrian Environment Authority conducted a scientific evaluation related to these events when the applicant originally applied for import only use. They found compelling scientific grounds not to warrant the approval of these events within its boarders (EFSA subsequently evaluated the Austrian submission, and yet rejected it on the basis that the report did not contain evidence that proved their assertions – inappropriately shifting the burden of proof on risks, not on safety). Their report (English follows the German summary) is submitted along with this assessment.

Second, it is worth noting that in a previous assessment for import use conducted by the VKM titled "Helse- og miljørisikovurdering av genmodifisert oljeraps – linje MS8, RF3 and MS8xRF3 fra Bayer CropScience AG (C/BE/96/01)", found that the applicants own toxicological data was out of date and not relevant for the evaluation of health consequences associated with use as food or animal feed2.

1. Insufficient information to support safe use related to inappropriate assumptions, faulty reasoning, or scientifically unjustified interpretations of the data by the Applicant

1.1 Inappropriate narrowing of the scope and analysis of application

It should be noted that the applicant has not supplied new data in relation to this application, merely analyzed existing data in relation to human and animal consumption. Further, the analysis is explicitly noted as limited in framing by the applicant. The Applicant perplexingly then concludes that "no new data has been found that impacts the conclusions of the previous risk assessments".

The Applicant states that:

"The scope of this application has been selected in order to cover accidental, unintentional presence of traces of MS8/RF3 oilseed rape grain in food. It complements existing scopes for the designated use of MS8/RF3 oilseed rape that have already been notified and authorized in the EU." (p. 6).

Yet we are unaware of any EFSA approvals or assessments that differentiates between levels of expected food use. Therefore we find the limitations by the applicant to conduct its analysis in a frame "to cover accidental, unintentional presence" in food as unjustified. If the applicant is to be granted approval for food use, there are no such restrictions as food under a limited circumstance. Hence, the applicant should conduct its analysis of safety on the basis the event would be wholly consumed as food and not consumed as a result of incidental presence in food.

The Applicant's interpretations on this basis can be found on p. of the dossier:

"Bayer CropScience AG is unaware of any additional data that would change the previous conclusions

of EFSA on the safety of MS8/RF3 oilseed rape or that would indicate that the previous conclusions cannot be extended to the scope of the current application, i.e. use of food containing or consisting of MS8/RF3 oilseed rape and food produced from MS8/RF3 oilseed rape or containing ingredients produced from MS8/RF3 oilseed rape (with the exception of processed oil) *with the aim to cover for accidental, unintentional presence of traces of MS8/RF3 oilseed rape grain in food*." Analyzing and the interpreting with such a narrow scope so narrowly may lead to the underestimation of potential exposure, and hence potential risks from the consumption of this event.

Recommendation:

The applicant's submission, and its interpretations should be conducted on the basis not only as "accidental, unintentional presence" in food, but as if the event applied for use in food was to be wholly consumed at any level of consumption. The application should be analyzed in such a manner that conforms not to the smallest level of exposure that would be allowed with the approval, but the largest.

1.2 Existing food safety/ exposure studies related to this application are insufficient to conclude safety of this product

1.2.1 Assessment of the study by Pfisher, 1999
We agree with the prior assessments of the VKM that the studies do not contain sufficient and/or relevant information to assess the safety of these events for human and animal consumption.

We find several fatal flaws in the aforementioned study used in support of a conclusion of safe
use. Specifically:

- Study design: Control and testing diets are insufficient to correlate the observed increase of lipids in blood. More test and control groups would be necessary to arrive at a scientifically supportable conclusion on elevated lipid levels.

- Study design: The groups of animals are too small to draw any statistical conclusions.

- Study design: Animal weights within groups are to variable to draw statistically meaningful conclusions. The rats used in the experiment are of different sizes at the beginning of the experiment, indicating they are likely from different age groups.

- Study design: Blood samples were taken just once after the feeding experiment. There is no baseline taken for comparison. The test animals were for 18h in a metabolic cage prior to when blood sample was taken, a high stress condition that may introduce secondary variables into the blood values.

- Missing information: The animals having PAT in the diet appear to have altered histology of the spleen, but no explanation given (only that changes were ”expected” in the strain of rats selected).

1.2.2 Assessment of the acute toxicity study of the BAR gene by Kennel, 2005.

Once again we find reason to agree with the prior assessments of the VKM that the studies referenced by the applicant do not contain sufficient and/or relevant information to assess the safety of these events for human and animal consumption.

We find several fatal flaws in the aforementioned study used in support of a conclusion of safe use. Specifically:

- Study design. 14 days are reported to have lapsed between injection of the test protein and histological and blood analyses. Shorter time intervals for analysis after injection would be necessary to capture shorter-term responses.

- Conclusions: The study authors place significant weight on the lack of mortality to conclude no toxic effect, yet clearly toxic effects do not necessarily need to be fatal to be acute. Only properly designed feeding studies using processed rapeseed and based on actual consumption patterns in the target population could clarify this point.

- Study design: Test protein used is not derived from plant-sources but a bacterial analog. Comparisions between feeding studies and direct test protein injection lacks scientific validity.

- Conclusions: The authors conclude that the protein is not allergenic based on the in silico study, yet immunogenic responses do not necessarily follow a dose-response curve.

Recommendation:
The applicant should submit newly designed toxicity and allergenicity studies relevant to the application at hand.

1.3 Lack of comprehensive exposure analysis

In their analysis, the applicant has only considered dietary exposure pathways in its assessment of possible adverse effects from MS8, RF3 AND MS8XRF3. Inhalation exposure can be expected to be a significant pathway for many people, and a more direct cause of potential adverse effects. The identified use of MS8, RF3 AND MS8XRF3 as a highly processed product, involves milling the grain to rapeseed flour. Humans may more likely have direct, non-dietary exposure to rapeseed flour than through dietary exposure, yet the applicant did not take this into account.

Inhalation experiment would provide possible direct lung cell exposure to any rapeseed flour, including MS8, RF3 AND MS8XRF3. Moreover, inhalation sensitization to allergens can be more important than dietary sensitization. In relation to soya exposure:

"It has to be considered that transgenic plants may be used in industrial processing; hence other exposure routes and sensitization scenarios might become important. For example, manufacturing large amounts of transgenic soybeans containing a food allergen may induce respiratory sensitization due to the generation of allergen-containing dust" (Spok et al., 2005).

Recommendation:
The applicant should provide information pertaining to the functional status of the transgenic protein after processing and also on the effects of MS8, RF3 AND MS8XRF3 inhalation in animals that are used as models of acute respiratory syndrome, compared with inhalation of the proper conventional comparator. This should include an analysis of allergenicity and toxicity.

Environmental risk assessment

The information provided by the applicant substantiates that Europe is the center of origin of oilseed rape, and it gives an important overview of the main oilseed rape producing areas world wide. From this overview presented in table 1p.11, it is evident that the EU countries and Europe as a whole, is the worlds main producing area of oilseed rape seed, oilseed rape oil and oilseed rape meal. The EU production of these commodities constitutes a high percent of the global total.

In section B 2. the applicant presents detailed figures for production and trade, giving oilseed rape seed imports into the EU by country of destination in 2012 season in table 15, p.88.. This shows imports of 1085.000 tonnes annualy to Belgium, 400.000 tonnes to France and 902.000 tonnes to the Netherlands. This is not necessarily evidence of high local use in those countries, but at least for Belgium and the Netherlands probably indicates that these countries harbour the main EU ports of importation of such bulk-material, to be distributed from there within EU. Given the substantial quantities and considerable distances and numerous logistical operations necessary for such bulk transport, spillage of viable seed will be unavoidable and we thus must enhance the need for sufficient environmental monitoring plans.

Information how the plant is typically cultivated, transported and stored, A.1.4.2 (p. 14)

The applicant presents the following referenced and substantiated information: *"In general, the oilseed rape in the EU is brought onshore by coasters or inland barges and unloaded to storehouses. From there it is transported to the crushing plant, where it is first cleaned and then pressed in a closed production process"* (A 1.4.2) Further in the same chapter the applicant states that; *"When rapeseed is imported for use in the oilseed crushing industry it is done in bulk and by shipping boats. While most seed is crushed in or near the ports of entry in the EU, a fraction of the imported viable seed can be transported inland to processing (crushing) facilities by boat, truck or rail (Devos et al., 2011)"* (A 1.4.2).

A closer reading of the mentioned source reveals the following additional information; *"The particular concerns related to feral GMHT oilseed rape fall within the range of general concerns stated above. They may cause a change in fitness, leading to invasion of semi-natural habitats, or to a colonisation of agricultural fields, where additional herbicide applications for weed control may be required due to the unintended stacking of HT traits. Feral GMHT oilseed rape plants may extend the potential for gene flow by acting as stepping stones and by forming populations that accumulate transgenes, thereby contributing to admixtures with commercially grown oilseed rape varieties. Based on such arguments, three EU Member States invoked national safeguard clause measures to provisionally ban the marketing of specific oilseed rape events on their territory"* (Devos et al., 2011).

We fully support this concern of the potential of herbicide tolerant varieties of oilseed rape to escape currently employed transport logistics and establish herbicide tolerant weedy

populations; having a competitive advantage due to the presence of genes conferring resistance to the most commonly used herbicides.

Such important questions relate to the importation, storage and within EU transportation of viable seed. These questions should be addressed in the environmental monitoring plan, which is a formal requirement in this application. Given the quantities of transgenic material to be imported, it is important to establish routines and systematic approaches within the logistics of storage and transportation, to avoid spillage and contamination. Typically such material is bulk-carried, with semi-open systems for handling and distribution.

Recommendation:
The applicant should take measures to ensure future coexistense of non-transgenic cultivars by eliminating the risk of both accidental spillage and contamination of transport equipment.

Social utility and sustainability aspects

In addition to the EU regulatory framework for GMO assessment, an impact assessment in Norway follows the Norwegian Gene Technology Act. In accordance with the aim of the Norwegian Gene Technology Act, production and use of the GMO shall take place in an ethically and socially justifiable way, under the principle of sustainable development. This is further elaborated in section 10 of the Act (approval), where it is stated that

> *"significant emphasis shall also be placed on whether the deliberate release represents a benefit to the community and a contribution to sustainable development"*.

These issues are further detailed in the regulation on consequence assessment section 17 and its annex 4. The Applicant has not provided relevant information that allows an evaluation of the issues laid down in the aim of the Act, regarding ethical values and social justification of the GMO within a sustainable development.
Given this lack of necessary information for a socio-economic evaluation, the Applicant has not demonstrated a benefit to the community or a contribution to sustainable development from the use of **MON88302xMS8xRF3 oilseed rape**. The Applicant should thereby provide the necessary data in order to conduct a thorough assessment on these issues, or the application should be refused.

Further, the Norwegian Gene Technology Act, with its clauses on societal utility and sustainable development, comes into play with a view also to health and environmental effects in other countries, such as where GMOs are grown. For instance, it is difficult to extrapolate on hazards or risks taken from data generated under different ecological, biological, and genetic contexts as regional growing environments, scales of farm fields, crop management practices, genetic background, interactions between cultivated crops, and surrounding biodiversity are all likely to affect the outcomes. Hence it cannot be expected that the same effects will apply between different environments and across continents.

Recommendation:
The applicant should submit required information on the social utility of **MON88302xMS8xRF3 oilseed rape** and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

Conclusion

Available information for risk assessment evaluation

This evaluation is based on the Applicant's own submitted information, along with our own expertise in related fields. The relevant scientific literature is very limited in some cases, yet we have tried to extract information from the peer-reviewed literature that may inform the scientific validity of the information under consideration. In situations where lack of knowledge, complexity and uncertainty are high, particularly in relation to unknown adverse effects that may arise as a result of approval for release of a living modified organism into the environment or food supply, the available information may not be sufficient to warrant approval. Further information may address some of these issues, however an accurate description of uncertainties provided by the applicant would provide a more useful basis for assessing the level of risk that may come with regulatory approval of the GMO, taken on a case-by-case basis.

In all cases, product-related safety testing should have an independent and unbiased character. This goes both for the production of data for risk assessment, and for the evaluation of the data.

The lack of compelling or complete scientific information to support the claims of the Applicant documented here highlights the need for independent evaluation of the dossier as performed here, including the raw data produced by the Applicant. We therefore support better transparency and independent review of information to ensure high standards within the regulatory process. This would include any information provided by the Applicant used to justify confidentiality claims on any scientific data. We encourage the authorities to insist on this level of transparency and accessibility to all scientific data (including raw data) to ensure the scientific validity of the information presented.

Overall recommendation

Above we highlight a number of issues in relation to the questionable safe use **MON88302xMS8xRF3 oilseed rape** that do not justify a conclusion of safe use, social utility and contribution to sustainable development. Critically, the Applicant's environmental monitoring plan lacks sufficient details and descriptions to support the required monitoring activities, and has not included any of the required information to assess social utility and sustainability as required in Appendix 4 of the Norwegian Gene Technology Act, which would be necessary for consideration of approval in Norway. Taken together, these deficiencies fail to address the necessary safety regulations under Norwegian Law, and thus the application is incomplete and should not be approved. A new application or reapplication should only be reconsidered with the delivery of the information requests recommended here, including any additional information deemed significant by the Norwegian authorities.

Therefore, in our assessment of **MON88302xMS8xRF3 oilseed rape** we conclude that based on the available data, the Applicant has not substantiated claims of safety satisfactorily to warrant approval in Norway at this time.

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